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Glossary

There is a fine line between words that provide necessary technical information and words that we might call *jargon*. Whereas technical vocabulary is important as a means to intellectual precision, jargon can often be used either to obscure ignorance or, like a badge, to identify members of an exclusive club. Both technical vocabulary and jargon, however, form a barrier between people already within a field of endeavor and those attempting to enter that field. Without identifying which of the following words are necessary and which are merely jargon, I include this somewhat selective glossary as an effort toward lowering that barrier.

Absorption: In the context of photochemistry, *absorption* refers to the utilization, by an atom or molecule, of light energy to raise electrons from their ground-state orbitals to orbitals at higher energy levels. Having absorbed the light energy, the atom or molecule is now in an excited state and will emit energy (in the form of either heat or light) when it returns to its ground state. Atoms will absorb light if, and only if, it is of a wavelength whose photons contain exactly the amount of energy separating a pair of electron orbitals within that atom.

Acquisition: In flow cytometry, *acquisition* refers to the process of recording the intensity of the photodetector signals from a particle in the transient memory of a computer. Once acquired, the data from a group of particles can be stored permanently on a storage medium from which it can be subjected to analysis. Acquisition and then analysis (in that order) are the two central steps in the flow cytometric procedure.

Acridine orange: Acridine orange (AO) is a stain that fluoresces either red or green, depending on whether it is bound to double-stranded or single-stranded nucleic acid. It has proved useful in comparing DNA and RNA content within cells; and it has also been used successfully (in the presence of RNase and mild denaturing conditions) to look at the changes in DNA denaturability during the cell cycle. There is debate in the flow cytometric community about whether the reputation that AO has for being difficult to work with is justified.

Activation marker: Activation markers are proteins that come and go on the surface of cells in response to stimulation. As such, they provide functional information about the physiological state of the cell. They also provide flow cytometrists with a reason to be concerned about instrument stability, sensitivity, and standardization.

ADC: An analog-to-digital converter converts photodetector signals (called *analog signals* because they are continuously variable, having an infinite variety of values) to channel numbers. The light intensity range represented by a given ADC channel depends on the amplification applied to the photodetector signal. ADCs can have 256 or 1024 or even 65,536 channels.

Aerosol: An aerosol is the spray of small fluid droplets that can be generated particularly when the nozzle of a flow cytometer is vibrated for sorting applications. If samples contain material that may be a biological hazard, attention should be paid to containment of the aerosol by suction through small-pore, hydrophobic filters.

Algae: Algae are simple forms of plant life. The larger algae are known as *seaweeds*. The unicellular algae form a large part of the plankton of both marine and fresh water environments and are suitable for analysis by flow cytometers. Algae also have contributed greatly to flow cytometric analysis because of their elaboration of pigments like phycoerythrin, PerCP, and allophycocyanin, which can be conjugated to antibodies and which facilitate multicolor staining procedures because they fluoresce in different regions of the spectrum.

Analysis: After acquisition, data from a sample are processed so as to provide useful results. This processing, or analysis, consists primarily of the correlation, in some or all possible ways, of the intensity channels from each of the signals recorded for each of the particles acquired for a given sample.

Analysis point: The three-dimensional volume in space where the laser beam intersects and illuminates the sample core of the fluid stream is the analysis point. The size of this point is determined by the cross-sectional dimensions of the laser beam and the width of the stream core itself. It is within the volume of the analysis point that particles are illuminated and signals are detected.

Aneuploid: Although *aneuploid* is used by cytogeneticists to refer to cells with abnormal numbers of chromosomes, it has been hijacked (with intellectual imprecision) by flow cytometrists to refer to the characteristic of possessing an abnormal amount of DNA. To be precise, flow cytometrists should use the term *DNA aneuploid* to acknowledge this distinction.

Annexin V: Annexin V is a molecule that binds to phosphatidylserine and, therefore, if conjugated to a fluorochrome, will identify apoptotic cells (which express phosphatidylserine on their surface). In the assay for apoptosis, annexin V must be used in conjunction with propidium iodide in order to exclude dead cells (which express phosphatidylserine on the internal side of their cytoplasmic membranes).

Apoptosis: Apoptosis is an ordered, active process that brings about the death of a cell as an important part of the maintenance of organismal homeostasis. Apoptosis can be assayed, in flow cytometry, by, for example, looking at the expression of phosphatidylserine on the cell surface, by looking for nuclei with less-than-normal (sub-G0/G1) amounts of DNA, and by looking for an increase in DNA fragment termini.

Arc lamp: An arc lamp is a device that emits light from a gas discharge between two electrodes. The wavelength of the light is determined by the gas used.

Autofluorescence: The light emitted naturally by an unstained, illuminated cell is called *autofluorescence*. The amount of auto-

fluorescence will differ depending on the type of cell, on the wavelength of the illuminating light, and on the fluorescence wavelength being analyzed. Autofluorescence, in general, results from endogenous compounds that exist within cells. It can be studied as an interesting phenomenon in itself; however, bright levels of autofluorescence at a particular wavelength will lower the sensitivity of a flow system for detecting positive stain of low intensity.

Back-gating: Back-gating is a strategy by which the fluorescence of stained cells within a mixed population is used to determine the side scatter and forward scatter characteristics of those cells. This is in contrast to “traditional” gating, whereby scatter characteristics are used to delineate cells whose fluorescence characteristics are then determined.

Beads: Beads are particles (made, usually, of polystyrene) that can be used as stable and inert standards for flow cytometric analysis. Beads can be obtained conjugated to various fluorochromes in order to standardize fluorescence detection settings and optical alignment or to calibrate fluorescence scales. They can also be conjugated to antibodies in order to calibrate the scale in terms of number of binding sites. More recently, in multiplexed assays, beads with capture molecules have been used to determine the concentration of soluble analytes.

BrdU: 5-Bromodeoxyuridine (also abbreviated BUdR or BrdUrd) is a thymidine analog that will be incorporated into the DNA of cycling cells. Cells pulsed with BrdU can then be stained with anti-BrdU monoclonal antibodies to indicate which cells have been synthesizing DNA during the pulse period. BrdU staining is a more precise way to look at the proportion of cells in S phase than simple propidium iodide staining for DNA content.

Break-off point: At the break-off point, some distance from a nozzle orifice, a vibrating stream begins to separate into individual drops that are detached and electrically isolated both from each other and from the main column of the stream.

Calcium: This ion’s rapid flux between cellular compartments and from the external medium is important in signal transduction

within cells. Its concentration can be measured by various fluorescent probes, such as fluo-3 and indo-1.

Channel: *Channel* is the term by which a flow cytometer characterizes the intensity of the signals emitted by a particle. Most cytometers divide the intensity of light signals into either 256 or 1024 channels. Signals with high channel numbers are brighter than signals with low channel numbers. However, the quantitative relationship between signals defined by one channel number and those defined by another will depend on the amplifier and photodetector voltage characteristics of a given protocol. Assignment of intensity to channel can be linear or logarithmic.

Coaxial flow: Coaxial flow is flow of a narrow core of liquid within the center of a wider stream. This type of flow is important in flow cytometry because it provides a means by which particles flowing through a relatively wide nozzle can be tightly confined in space, allowing accurate and stable illumination as they pass one by one through a light beam.

Coherence: Coherence is the property of light emitted from a laser such that it is remarkably uniform in color, polarization, and spatial direction. Spatial coherence allows a laser beam to maintain brightness and narrow width over a great distance.

Coincidence: Coincidence, in flow cytometry, is the appearance of two cells or particles in the laser beam at the same time. The flow cytometer will register these two cells as a single event (with approximately twice the fluorescence intensity and light scatter as a single cell). To avoid coincidence, the concentration of cells in the sample should be low, the laser beam should be small in the direction of flow, and the sample core should be narrow.

Compensation: Compensation is the way a flow cytometrist corrects for the overlap between the fluorescence spectra of different fluorochromes. Without compensation, fluorescence from a given fluorochrome may be included to some extent in the intensity value coming from a photodetector assigned to the detection of a different fluorochrome. Compensation can be electronic or can be applied by software during analysis of stored data.

Contour plot: A contour plot is one method for displaying data cor-

relating two cytometric parameters. The density of particles at any place on the plot is used to generate contour lines (much as contour lines on a topographic map are used to describe the height of mountains at different points in the landscape). The particular algorithm used to generate the contour lines can make a great difference in the visual impact of the display.

Control: A control is a type of sample that, most particularly in flow cytometry, you generally have one fewer than you actually need.

Core stream: The core stream is the stream-within-a-stream that has been injected into the center of the sheath stream and is maintained there by the hydrodynamic considerations of laminar flow at increasing velocity. The core contains the sample particles that are to be analyzed in the flow cytometer. If the sample is injected too rapidly, the core stream widens and particles may be unequally illuminated. In addition, with a wide core stream, coincidence events are more likely.

Coulter volume: A cell's Coulter volume is the increased impedance that occurs as the cell displaces electrolyte when it flows through a narrow orifice. This increase in impedance is related to the volume of the cell.

Cross-match: Cross-matching is the process of testing the cells of a prospective organ donor with the serum of a prospective organ recipient for compatibility. The flow cytometric cross-match determines whether serum from the recipient contains antibodies that bind to the donor cells. Such binding constitutes a positive cross-match and is a contraindication to transplantation in that particular donor/recipient combination.

Cross-talk: Cross-talk is the signal from the "wrong" photodetector that results because the fluorescent light emitted by one fluorochrome contains some light of a wavelength that gets through the filters on a photodetector that is nominally specific for the fluorescence from a different fluorochrome. See **Compensation**.

CV: The coefficient of variation (CV) is defined as the standard deviation of a series of values divided by the mean of those values (expressed as a percentage). It is used in flow cytometry to describe the width of a histogram peak. Whereas in some proto-

cols it can be used to assess the variation in particle characteristics within a population, in DNA analysis (where all normal particles are assumed to have identical characteristics) it is frequently used to assess the alignment of a flow cytometer (and the skill of its operator). Discussions about CV have been known to bring out rather primitive competitive instincts within groups of flow cytometrists, who are usually friendly, well-adjusted people.

Cytofluorimeter: *Cytofluorimeter* is a term synonymous with *flow cytometer*, but with slightly antiquated overtones.

Deflection plates: Deflection plates are the two pieces of metal that carry a high voltage to attract charged drops to one side or the other of the main stream. It is by means of the charge on the deflection plates that sorting of particles occurs. The deflection plates also provide a good reason for flow cytometry operators to keep their hands dry and wear rubber-soled shoes.

Detergent: Detergents, such as saponin, Triton X-100, or NP40, are used to aid in the permeabilization of cell membranes in order to facilitate staining of intracellular proteins.

Dichroic mirror: A mirror that by virtue of its coating reflects light of certain wavelengths and transmits light of other wavelengths is a dichroic mirror. Such mirrors are commonly used in flow cytometry at 45° to the direction of incident light in order to transmit light from specific fluorochromes to a particular photo-detector and reflect light from different fluorochromes toward different detectors. The exact angle at which the mirror is positioned affects its wavelength specificity.

Dot plot: A dot plot is a two-dimensional diagram correlating the intensities of two flow cytometric parameters for each particle. Dot plots suffer, graphically, from black-out in that an area of a display can get no darker than completely black. If the number of particles at a given point is very dense, their visual impact, in comparison with less dense areas, will decrease as greater numbers of particles are displayed. Contour plots display the same kind of correlation as dot plots but, because the levels of the lines can be altered, can provide more visual information about

the density of particles at any given point in the correlation display. Dot plots can also be called *two-dimensional histograms*.

Drop delay: In flow sorting, the drop delay is the time between the measurement of the signals from a particle and the moment when that particle is just about to be trapped in a drop that has broken off from the main column of a vibrating stream. It is at this precise moment that the column of the stream must be charged (and then shortly thereafter grounded) if the drop containing a particle of interest is to be correctly charged so that it can be deflected to the left or right out of the main stream.

Emission: Emission is the loss of energy from an excited atom or molecule in the form of light. Although a long-lived form of light emission (known as *phosphorescence*) does occur, in flow cytometry the light emission with which we are primarily concerned occurs rapidly after excitation and is called *fluorescence*.

Erythrocyte: The red, hemoglobin-containing cells that occur in the peripheral circulation and are responsible for transporting oxygen are erythrocytes. Despite this vital physiological function, immunologists are apt to feel a certain amount of hostility toward erythrocytes because they outnumber white cells by about 1000 to 1 and therefore make flow analysis of white cells difficult unless they are removed by either lysis or centrifugation. Fetal erythrocytes are, conversely, of great interest when they appear in the maternal circulation because they can be used for prenatal screening and for diagnosis of fetal–maternal hemorrhage. When fixed in glutaraldehyde, chicken or trout erythrocytes fluoresce brightly at a broad range of wavelengths and are therefore a useful tool for aligning the cytometer beam; when fixed in ethanol, they are a useful DNA standard (containing less DNA than a normal human cell).

Euploid: Flow cytometrists use the term *euploid* to refer to a cell with the “correct” amount of DNA for its species. Because its sensitivity is limited and because it works only with total DNA content, a flow cytometer may not agree with a microscopist (who looks at chromosomes) in the classification of cells by this criterion. See also **Aneuploid**.

Event: An event is the name given by flow cytometrists to what most people would call a cell. A flow cytometer associates all light signals that occur without a gap in time with a single event and stores the intensities of the light in association with that event in the data file. If cells (or other particles) are spaced appropriately in the core stream and do not coincide in the laser beam, then an event is the same as a cell or particle. If cells do coincide in the laser beam, then an event may be two or more cells.

FACS: FACS is an acronym for *Fluorescence-Activated Cell Sorter*. It is a term coined by the Herzenbergs at Stanford University and used by Becton Dickinson for its instruments, but has come to be used generally as a term that refers to all instruments that analyze the light signals from particles flowing in a stream past a light beam. The term *flow cytometer* is perhaps more correct because it has neither the trademark connotations nor any reference to a sorting function, which most of these instruments no longer possess. The term *cytofluorimeter* is also used, but generally has antiquated overtones.

FCS format: *Flow Cytometry Standard* is a file format for flow cytometric data storage. Adherence to this format facilitates the programming for analysis of results. Manufacturers of cytometers have finally begun to embrace this standard.

Fetal hemoglobin: Fetal hemoglobin (HbF) is a form of hemoglobin that exists primarily in the erythrocytes of the fetus before birth. Because it has higher oxygen affinity than adult hemoglobin, it aids in the transfer of oxygen from the maternal to the fetal circulation. Antibodies against HbF can be used to detect fetal erythrocytes. By staining for fetal erythrocytes in the maternal peripheral blood, flow cytometry can be used to diagnose fetal–maternal bleeding across the placenta and also (in conjunction with DNA staining) to sort rare nucleated fetal erythrocytes for prenatal diagnosis of disease.

Filter (1): A glass filter (either colored glass or interference) modifies the light that passes through it. A neutral density filter reduces the intensity of a light beam without affecting its color. Short-pass, long-pass, and band-pass filters selectively alter the color of a light beam by transmitting light of restricted wavelength. If

positioned at a 45° angle to the incident light, these filters are called *dichroic mirrors* (see above). The specifications of a band-pass filter are given in terms of the wavelength of the maximally transmitted light and the band width (in nm) at half-maximal intensity.

Filter (2): A paper or nylon filter removes particles above a certain size from liquid. In flow cytometry, these filters are important for cutting down on background noise by removing small particles (<0.22 µm) from the sheath fluid and also for removing clumps from your sample (<35 µm).

FITC: See **Fluorescein**.

Fixation: Fixation is the process by which the protein of cells is denatured, or cross-linked, and preserved. Fixation in flow cytometry is used to inactivate hazardous biological material and also to preserve stained cells when there is not immediate access to a flow cytometer. Fixation is also important in preserving proteins before detergent permeabilization for intracellular staining. Formaldehyde is often the fixative of choice for flow cytometry because it preserves the forward and side scatter characteristics of cells (but does cause some increase in their autofluorescence).

Flourescence: A common misspelling of *fluorescence* often found in keyword listings for articles on flow cytometry.

Flow: *Flow* is a term that has come to refer, colloquially, to the general technique of flow cytometry. For example “Do you use flow to analyze your cells?” or “Flow has revolutionized the study of immunology” or “I’m off to do flow.”

Flow cell: The flow cell is the device in a flow cytometer that delivers the sample stream to the center of the sheath stream and then accelerates the flow velocity to maintain the cells spaced out from each other, in a narrowing core. In some cytometric configurations, the laser illuminates the stream within the flow cell; in other configurations the illumination occurs “in air” after the stream has left the flow cell. In the latter case, the term *nozzle* is more apt to be used. In a sorting instrument, the flow cell vibrates in order to allow drop formation. It also provides

the electrical connections for charging and then grounding the stream at appropriate times.

Flow cytometry: See Chapters 1 through 12 of this book.

Flower: *Flower* is a term of affection in the Northeast of England—and one that could, with a slight change of pronunciation, be adapted to refer to a person who works in the field of flow cytometry.

Fluorescein: Fluorescein is a fluorescent dye that can be readily linked to proteins and that is therefore useful, when conjugated to specific antibodies, for lighting up cells with particular phenotypes. It is sometimes abbreviated as FITC because fluorescein isothiocyanate is the chemically active form of the molecule that is used in the conjugation process.

Fluorescence: Fluorescence is a form of light emitted by atoms or molecules when electrons fall from excited electronic energy levels to their lower, less-energetic ground state. *Fluorescence* is often misspelled as *flourescence*, so it pays to check both spellings when doing a key-word literature search.

Fluorochrome: A fluorochrome is a dye that absorbs light and then emits light of a different color (always of a longer wavelength). Fluorescein, propidium iodide, and phycoerythrin, for example, are three fluorochromes in common use in flow cytometry. *Fluorophore* is an equivalent term.

Fluorogenic substrate: A fluorogenic substrate is a nonfluorescent chemical that is an enzyme substrate and that yields a fluorescent product when processed by the enzyme. A fluorogenic substrate can therefore, by virtue of its increasing fluorescence, be used in a flow cytometer to measure the activity of a given enzyme within a cell. Fluorescein digalactopyranoside (FDG) is a fluorogenic substrate for the enzyme β -galactosidase.

Forward scatter: Forward scatter is light from the illuminating beam that has been effectively bent (refracted or otherwise deflected) to a small angle as it passes through a cell so as to diverge from the original direction of that beam. The intensity of the light bent to a small angle from the illuminating beam is related to

the refractive index of the cell as well as to its cross-sectional area. The forward scatter signal causes a great deal of confusion because some people who call it a *volume* signal actually begin to think that it is closely correlated with a cell's volume. *Forward scatter* is often abbreviated as FSC or as FALS (for “forward angle light scatter”).

G0/G1: In flow cytometry, the term refers to cells that have the 2C (diploid) amount of DNA and are therefore either not cycling (G0) or have just completed cytokinesis and have not yet begun again to make more DNA in preparation for a new cycle (G1).

G2/M: In flow cytometry, the term refers to cells that have the 4C (tetraploid) amount of DNA and are therefore either just completing DNA synthesis in preparation for mitosis (G2) or are involved in the steps of mitosis before cytokinesis (M).

Gain: Gain is a measure of the amplification applied to a signal. In flow cytometry, it occurs between the time of the initial detection of a light pulse and the final output that is stored as the channel on an ADC (and then in a data file). For a weak light pulse, the gain can be increased by the use of an amplifier, increasing the electrical signal after it leaves the photodetector; or it can be increased by altering the voltage directly applied to a photomultiplier tube.

Gate: Gate is a restriction placed on the flow cytometric data that will be included in subsequent analysis. A *live gate* restricts the data that will be accepted by a computer for storage; an *analysis gate* excludes certain stored data from a particular analysis procedure; a *sort gate* defines the cells that will be selected for sorting. See also **Back-gating**.

GFP: Green fluorescent protein comes from the jellyfish *Aequorea victoria*. It fluoresces green, which is of puzzling utility to the jellyfish but of great use to scientists because it can act as a reporter molecule or can be used to make fusion proteins visible. Outdoing the jellyfish, molecular biologists have created GFP analogs that fluoresce in different colors (BFP, CFP, and YFP).

Granularity: *Granularity* is a term sometimes used synonymously with *side scatter* to describe the light that is deflected to a right

angle from the illuminating beam in a flow cytometer. The intensity of this light is related, in an imprecise way, to internal or surface irregularities of the particles flowing through the beam.

Granulocyte: Granulocytes are the major constituents of a class of white blood cells (called *polymorphonuclear cells*) that possess irregular nuclei and cytoplasmic granules and therefore have bright side scatter signals.

Hard drive: A hard drive is a storage medium for software and for data. Hard drives have relatively large memory capacity but, when not removable, require further back-up facilities because they will always become filled faster than you imagine.

Heath Robinson device: W. Heath Robinson (1872–1944) was an English humorous artist. A *Heath Robinson device* is the English term for a complex instrument that looks as if it had been designed by a committee. A Heath Robinson device usually works well but may be held together by chewing gum and string. In the context of state-of-the-art flow cytometry, this term requires no further explanation. See also **Rube Goldberg device**.

High-speed sorting: High-speed sorting is a flow cytometric technique whereby adaptations to pressure and fluid controls allow high stream velocities and rapid drop generation. This decreases the probability of multiple cells in a single drop and consequently increases cell-recovery efficiency when sorting at fast rates. Such adaptations are important when large numbers of relatively rare particles are required.

Histogram: A one-dimensional histogram displays data from one parameter of a flow cytometric data file at a time.

Hydrodynamic focusing: Hydrodynamic focusing is the property of laminar flow in a stream of increasing velocity that maintains particles in the narrowing central core of a column of fluid.

Immunophenotyping: Immunophenotyping is the classification of normal or abnormal white blood cells according to their multi-parameter surface antigen characteristics.

Interrogation point: *Interrogation point* is synonymous with *analysis point*.

Laser: Laser is an acronym for *Light Amplification by Stimulated Emission of Radiation*. Lasers are important in flow cytometry because, as a result of their coherent output, they are a means of illuminating cells with a compact, intense light beam that will produce fluorescence signals that are as bright as possible over a short time period.

Lens: A lens is a means of changing the shape of a beam of light. In flow cytometry, lenses are used to narrow the laser beam to a small profile at the stream. Some lenses produce a beam with a circular cross-sectional shape; others produce beams with an elliptical configuration. Lenses are also used in a flow cytometer to collect scattered light and fluorescence and then to transmit them to an appropriate photodetector.

Linear amplifier: A linear amplifier is one means of increasing the signal from a photomultiplier tube to make it stronger. A linear amplifier increases the signal in such a way that the output voltage from the amplifier is directly proportional to the input current derived from the photodetector. Linear amplifiers are used, in particular, for signals from the DNA of cells because the range of intensities being studied is small. See also **Logarithmic amplifier**.

List mode: List mode is a method of data storage in which the intensities of the signals from each photodetector that are generated by a single particle are stored in association with each other so that they act as a total flow cytometric description of that particle. A list mode data file consists of a long list of numbers, describing each cell in the order in which it passed the laser beam. The descriptive numbers from each particle can be correlated with each other (and with the descriptive signals from all other particles) in all possible ways. If you really want to understand the meaning of “list mode,” you should ask Howard Shapiro to sing his song of that name.

Logarithmic amplifier: Logarithmic amplification is one means of modifying the signal from a photomultiplier tube. A logarithmic amplifier modifies the signal in such a way that the output voltage from the amplifier is in proportion to the logarithm of the input current derived from the photodetector. Logarithmic am-

plifiers are conventionally used for fluorescence signals from cellular proteins, where the range of signals (covering stained and unstained particles) is large. See **Linear amplifier** for comparison.

Lymphocyte: A lymphocyte is a particular type of white blood cell that is involved in many of an organism's immune responses. Subpopulations of lymphocytes with microscopically identical anatomy can be distinguished because their surface membranes contain different arrays of proteins. The staining of these proteins with fluorescently tagged monoclonal antibodies allows the subpopulations to be enumerated by flow cytometry.

Marker: *Marker* is a term that is often used to refer to a dividing line applied to a fluorescence intensity histogram in order to dichotomize particles into those that are to be called positively stained from those to be called unstained. Somewhat confusingly, it is also used by immunologists to refer to a significant protein on the surface of a cell.

Mean: The mean is a value that can be used to describe the fluorescence intensity of a population of cells. The arithmetic mean is calculated by adding up the intensities of n cells and then dividing that sum by n . It can be skewed considerably by the presence of a few very bright or very dim cells. The geometric mean is calculated by multiplying the intensities of n cells and then deriving the n th root of that product. It is less affected by outliers, but, accordingly, will not compare well with biochemical analysis. The calculation of a mean from a flow histogram will incorrectly evaluate any particles that lie in the highest and lowest (i.e., off scale) channels. See **Median** and **Mode**.

Median: Median is a value that can be used to describe the fluorescence intensity of a population of cells. If the cells were lined up in order of increasing intensity, the median value would simply be the channel number or intensity of the cell that is at the midpoint in the sequence. The median channel, because it is unaffected by off-scale events and outliers, is considered by many to be the best way to describe the intensity of a population. See **Mean** and **Mode**.

Mode: Mode is a value that can be used to describe the fluorescence intensity of a population of cells. The mode intensity is the channel number that describes the largest number of cells in a sample. Mode values are apt to be variable when intensity distributions are broad or when few particles have been analyzed. See **Mean** and **Median**.

Monocyte: Monocytes are a class of white blood cells that co-purify with lymphocytes in commonly used density gradient procedures. They tend to be promiscuously sticky for the nonspecific (Fc) ends of monoclonal antibodies and therefore can lead to misleading results in analysis of leukocyte subpopulations unless their Fc receptors are blocked in the staining procedure. Monocytes differ from lymphocytes in their forward and side scatter characteristics.

Necrosis: Necrosis is a cell's response to overwhelming injury. It has been referred to as "accidental cell death" by way of comparison with **apoptosis**, which is considered to be a more orderly and beneficial process. The flow cytometric hallmark of necrosis is permeabilization of the outer membrane (leading to propidium iodide fluorescence).

Nozzle: See **Flow cell**.

Obscuration bar: A forward scatter obscuration bar is a strip of metal or other material that serves to block out direct light from the illuminating beam. Any light reaching a photodetector will therefore be light that has been deflected around the bar by the physical characteristics of a particle interacting with the light. The width of the bar will define the narrowest angle by which the light must be deflected in order to reach the detector. In jet-in-air systems, there is also a side scatter obscuration bar, which decreases the background noise in the side scatter channel from laser light deflecting to wide angles from the stream-air interface.

Observation point: See **Analysis point**.

Optical bench: An optical bench is the stable table ;) that keeps the light beam, fluid streams, lenses, and photodetectors of a flow cytometer all precisely aligned with each other. Lack of stability in these components leads to artifactual results.

Optical sorting: Optical sorting is a flow method for deactivating cells or chromosomes by use of an additional (zapper) laser in conjunction with phototoxic dyes. Because the zapping laser can be deflected and returned to the flow stream at a rapid rate, optical sorting has the potential for being more rapid than traditional or high-speed droplet sorting.

Orifice: The orifice is the exit opening in a flow cell or nozzle. Its diameter can, in general, range from 50 to 300 μm . Cells or aggregates of cells anywhere near as large as the orifice will block it. The diameter of the orifice affects the possible drop frequencies for flow sorting.

Parameter: *Parameter* is the term applied to the types of information derived from a cell as it goes through the flow cytometer. The number of parameters measured by a cytometer is determined by the number of photodetectors present and also by any processing of the signals from each photodetector to provide “derived” parameters like signal area or signal width. Modest cytometers measure three parameters. Immodest cytometers measure 13 parameters. Average cytometers measure between four and six parameters. In the future, our standards may increase.

Particle: Particles are the objects that flow through flow cytometers. It is a general term that includes cells and chromosomes and beads and DNA fragments and gel microdrops, among others.

Peak reflect: The peak reflect method is an algorithm for analyzing DNA histograms to determine the number of cells in the S phase of the cell cycle. In the peak reflect method, the shapes of the 2C and 4C peaks are assumed to be symmetrical, thus allowing subtraction of the contribution from these two peaks from the S-phase cells between them.

Phase sensitivity: A phase-sensitive flow cytometer quantifies the life time of the fluorescence emitted by particles. The decay time of fluorescence from a given fluorochrome is altered by changing chemical environments, and therefore measurement of fluorescence lifetimes can provide information about the microenvironment surrounding the fluorescent probe.

Photodetector: A photodetector is a device that senses light and converts the energy from that light into an electrical signal. Within the operating range of the detector, the intensity of the electrical signal is proportional to the intensity of the light. Photomultiplier tubes and photodiodes are two types of photodetectors.

Photodiode: A photodiode is a type of photodetector used to detect relatively intense light signals. It does not have a high voltage applied to increase the current flow at its anode (output) end.

Photomultiplier tube: A photomultiplier tube is a type of photodetector used to detect relatively weak light signals. Its output current is increased by means of high voltage applied to it.

Phycoerythrin: Phycoerythrin is a fluorochrome derived from red sea algae. It is particularly useful in flow cytometric applications requiring dual-color analysis because, like fluorescein, it absorbs 488 nm light from an argon laser. However, it has a longer Stokes shift than fluorescein, and therefore the fluorescences of the two fluorochromes can be distinguished.

Plankton: Plankton are the small organisms that float in lakes and oceans and are moved passively, drifting with the tides and currents. Small plankton are excellent objects for flow cytometric analysis.

Precursor frequency: The precursor frequency of cells that have divided in response to a stimulus is the proportion of cells present at the time of the addition of the stimulus that were destined to begin division. It is a measure of the potential of a mixed population of cells for response to any particular activator and can be determined, by flow cytometry, with the use of fluorescent tracking dyes that stain cells with stability but dilute by half each time a cell divides.

Probe: *Probe* is a general term used, in flow cytometry, to refer to any chemical that fluoresces when it reacts or complexes with a specific class of molecules and therefore can be used to assay that molecule quantitatively. Propidium iodide and acridine orange are probes for nucleic acid because they complex specifically with nucleic acids and fluoresce brightly when they have

reacted in this way. Fluo-3 is a calcium probe because it chelates calcium ions and fluoresces brightly when it is complexed with this ion.

Propidium iodide: Propidium iodide is a probe that can be used to measure quantitatively the amount of double-stranded nucleic acid that is present in a cell. After treatment of cells with RNase, it will measure the amount of DNA present. Because it does not cross an intact cell membrane, cells need to be treated with detergent or ethanol before it can be used to determine their DNA content. It can also be used to assess the viability of cells.

Quadrant: For dual-parameter analysis, a two-dimensional plot of particles according to the correlated fluorescence intensities of two colors is, traditionally, divided into four quadrants. The division is based on the background fluorescence of the unstained control sample. The quadrants, from this division, will contain (1) cells that have stained with the first fluorochrome only; (2) cells that have stained with both fluorochromes; (3) cells that remain unstained; and (4) cells that have stained with the second fluorochrome only. In other words, the quadrants are defined so that they delineate the two types of single positive cells (1 and 4), double-negative cells (3), and double-positive cells (2).

Rare event: Defining a rare event in flow cytometry is a bit like answering the question “How long is a piece of string?” Particles that are less than 0.1% of the total are considered somewhat rare and require some care for enumeration in a flow cytometer. Really rare particles are less than 0.001% of the total and can be detected, counted, and sorted with careful gating based on multiparameters.

Region: A region is a description, using flow cytometric characteristics, that will define a cluster of cells. A region, in the old days, was always rectangular—with an upper and lower channel limit for each parameter involved in the description. Now, a region can take any shape, based on the channel numbers defining all the vertices of an irregular polygon or ellipse. Multiple regions can be combined by Boolean logic (AND, OR, or NOT) to define a gate that is used for cell analysis. See **Gate**.

Rube Goldberg device: Reuben Lucius Goldberg (1883–1970) was an American humorous artist. A *Rube Goldberg device* is the American synonym for a Heath Robinson (see above) device. Mr. Goldberg and Mr. Robinson may or may not have known each other, but surely would have enjoyed each other's company.

S-FIT: An S-FIT approximation is a mathematical algorithm for guessing at which cells in a DNA-content histogram are actually in the S phase of the cell cycle. The S-FIT algorithm bases this guess on the shape of the DNA histogram in the middle region between the G0/G1 and the G2/M peaks.

S phase: S phase is the period of the cell cycle during which cells are in the process of synthesizing DNA in preparation for cell division. During S phase, cells have between the 2C amount of DNA normal to their species and the 4C amount of DNA, which is exactly double the 2C amount. It is the overlap of fluorescence intensity between cells in S phase and some of the cells with the 2C and 4C amounts of DNA that leads to uncertainty in the flow cytometric estimation of the S-phase fraction.

Sheath: Sheath is the fluid within which the central sample core is contained during coaxial flow within and from the flow cell of a flow cytometer.

Side scatter: Side scatter is light of the same color as the illuminating beam that bounces off particles in that beam and is deflected to the side. The “side” is usually defined by a lens at right angle (orthogonal orientation) to the line of the laser beam. Side scatter light (SSC) may alternatively be called *right-angle light scatter* (RALS) or *90° LS*. The intensity of this light scattered to the side is related in a general way to the roughness or irregularity or granularity of the surface or internal constituents of a particle.

Signature: *Signature* is a term used by marine flow cytometrists to refer to the flow cytometric characteristics of particular planktonic species in a sample of water. The signature of plankton is related, in particular, to the autofluorescent pigments that are present. *Signature* has also been used to describe the distin-

guishing histogram profile that results from the DNA-fragment analysis of different bacterial species.

SOBR: SOBR is a mathematical algorithm for guessing at which cells in a DNA-content histogram are actually in the S phase of the cell cycle. The SOBR (sum of broadened rectangles) algorithm bases this guess on the use of Gaussian-broadened rectangular distributions to attempt to fit the shape of the DNA fluorescence intensity histogram of the cells in question.

Stokes shift: The Stokes shift is the difference in color (expressed as either energy or wavelength) between the light absorbed by a fluorochrome and the light emitted when that fluorochrome fluoresces.

Tandem dye: Tandem dyes are conjugates of two different fluorochromes. They use resonance energy transfer to pass the light energy absorbed by the primary fluorochrome to the secondary fluorochrome, which then emits that energy as fluorescence. Tandem dyes occur naturally (e.g., the peridinin:chlorophyll complex or the aequorin:GFP complex) and can also be manufactured by organic chemists (e.g., PE-Cy5). In flow cytometry, tandem dyes are useful because they effectively increase the Stokes shift of a fluorochrome and thereby allow more fluorescence parameters to be observed with a single excitation wavelength.

Tetraploid: *Tetraploid* is a term used to describe cells with double the amount of DNA normal for a particular species. Malignant cells are frequently of the tetraploid type. Distinguishing this type of ploidy from normal G2 or M cells or, indeed, from clumps of two cells can be difficult with flow cytometry.

Threshold: The threshold is an electronic device by which an ADC can be made to ignore signals below a certain intensity. A forward scatter threshold is most commonly used in flow cytometry to exclude very small particles, debris, and electronic or optical noise from acquisition into a data file.

Time: Time is a parameter that is being used more frequently in conjunction with flow cytometric analysis. It is being used in relation to the functional analysis of cells for the rate of reaction

(e.g., calcium ion flux or changes in membrane potential) in response to various stimuli. It is also being used, on a finer scale, in phase-sensitive measurements of fluorescence decay.

TUNEL: The TUNEL assay represents a historical low point in attempts to coin acronyms. The *Terminal deoxynucleotidyl transferase-mediated dUTP Nick End-Labeling* assay labels the ends of DNA with fluorescent UTP. Apoptotic cells often have fragmented DNA; these fragments will provide more substrate for the enzyme terminal deoxynucleotidyl transferase. Apoptotic cells can therefore be enumerated by flow cytometry according to their increased intensity in the TUNEL assay.

Volume: Volume is a useful and definite characteristic of any particle—but one that is not easily amenable to flow cytometric analysis. “Coulter volume” does measure volume to a close approximation, but “forward scatter” does not.

Wavelength (1): Wavelength is a characteristic of light that is related exactly to its energy content and also (with light to which our eyes are sensitive) to its color. Light of short wavelength lies toward the bluer region of the spectrum and has more energy than light of longer wavelength. Wavelength is used to describe the characteristics of filters, of dichroic mirrors, of laser beams, and of the absorption and emission of fluorochromes.

Wavelength (2): In sorting flow cytometry, *wavelength* is a term used to describe the distance between drops as they form from a vibrating fluid jet. This drop wavelength is determined by the diameter of the stream as well as by its velocity.